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Sensitivity of Elisa for Hepatitis C Virus Identification in Serum and Comparison of Elisa with ICT

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ABSTRACT

In the developing countries, Immuno Chromatographic Techniques (ICT) are used instead of ELISA which may give false positive and false negative results. But ELISA technique has more than 99% accuracy, sensitivity and specificity regarding the diagnosis of Hepatitis C Virus. A cross sectional study on 200 patients is the part of this study to check sensitivity of ELISA for HCV as well as to compare the sensitivity of ELISA and ICT method for HCV. For investigation of Pakistani patients using these techniques, 50 samples of control group (25 samples of negative control and 25 of positive control) and 150 samples of patients (suspected for HCV) were collected. Their HCV tests were first performed by using ICT kits. Then these samples were tested for anti-HCV antibody through ELISA by fully automated Instrument and their cut off values was determined. Results obtained by ELISA were compared by the results obtained by testing through ICT method. Results revealed that twenty-five patients suffering from hepatitis C were detected by both ELISA and ICT (positive control). Similarly, ELISA and ICT showed the negative results for all twenty-five negative control patients. Both ELISA and ICT showed the same results regarding detection of hepatitis C except two patients in experimental group. ICT showed positive result of a patient who was healthy and also confirmed negative by ELISA technique. Also this patient did not show any sign and symptom of hepatitis C. Further, ICT showed negative result of hepatitis C infected patient who has the sign and symptoms of hepatitis C and also ELISA showed positive result regarding detection of hepatitis C. It can be concluded from this experiment that both ELISA and ICT can be used for the detection of hepatitis C. However, the sensitivity of ELISA is higher as compared to ICT. Therefore, it can be recommended that ELISA is more appropriate method for the detection of hepatitis C.

Keywords: *ELISA, Diagnosis, ICT, Antibody, Appropriate, Symptoms.*

1. Introduction

Laboratory tests which are used in diagnosis of hepatitis include following routine tests: CBC, LFTs including albumin, alkaline phosphatase, alanine amino transferase and bilirubin and aspartate amino transferase. There are serologic tests including immunoassays for antigens and antibodies to perceive that infection is present or not, the disease status, the immune status, and requirement of treatment. ICT and Enzyme linked immunosorbent assay ELISA are Immuno assays used for HCV. Tests based on Nucleic acid are utilized to detect viremia and viral load. Hepatitis genotype is determined by Genotyping tests.

When cells of liver are damaged and have swelling then primary tests which are used to detect these changes are alanine transaminase and aspartate transaminase. There is another enzyme named alkaline phosphatase that can be elevated in such liver impairment. When both ALT and AST are compared then ALT is more specific comparatively than AST. Therefor it is used for assessing response to treatment. When there is acute form of hepatitis then level of ALT is elevated 10 times to that of normal level. This value is constant for almost 2 months. Then it starts decreasing and begins to normal with in duration of 6 months. But if there is chronic infection by HCV then ALT level is not elevated markedly in serum. It can be about 4 times to normal level of serum. Several drugs have effect on serum level of ALT so it is important to ask about all used drugs prescribed or un-prescribed by the patients before testing for ALT.

When liver is damaged by the virus then proteins which are synthesized by the liver, tend to decrease in serum showing that liver is not able to prepare these in serum. So level of albumin and total protein is decreased in serum.

Bilirubin levels are elevated because liver is not able to filter this bilirubin from blood. It continues to enter in blood from destruction of RBCs. A liver biopsy is used in some cases if decision cannot be made from above mentioned tests to detect that how much severe is infection of liver (Ghany and Strader, 2009). ICT, ELISA, RIBA are immunological assays used for detection of HCV infection. Quantitative and qualitative PCR are the molecular assays. Examination of liver biopsy test is ELISA assessments are high-priced as the chemicals and instruments are needed for performance of test however window period of HCV is shortened in this method. RNA of HCV can be amplified through the use of Nucleic Acid Amplification Technology as properly. It is more sensitive and it shortens the window period to four days. Polymerase chain reaction (PCR) may be used to detect HCV RNA to discover acute infection caused by hepatitis C virus. PCR is high cost test as it requires trained team of workers and special understanding. ELISA and PCR have enhanced ranges of microbiological assays that are diagnostic over the last 30 years. These methods pioneered by Pasteur about in the last 19th century are still used to diagnose the infectious diseases.

The aim of treatment is to avoid complications caused by HCV infection. This is possible if infection is eradicated. HCV RNA testing is used to assess treatment response. If there is a sustained virologic response (SVR) then infection is considered to be eradicated. SVR is defined as the nonappearance of RNA of HCV in serum using a sensitive test six months later and at the end of treatment. Persons those who attain an SVR almost have a sudden decrease in RNA level of HCV. If virus is continuously not detected when treatment is terminated, then it is called end of treatment response. Constant absenteeism of visible virus at termination of treatment is referred to as end of treatment response. If patients have such condition that HCV RNA is not detected when he is on treatment but again HCV RNA is detected when treatment is stopped. Then patient is said to have relapses. But if HCV RNA levels in patient serum remain constant then patient is said to as non-responder. Some patients are partial responder's means that level of HCV RNA continuously decreases but is not undetected.

Patients who are taking pegylated interferon (peg interferon) or interferon or in combination with ribavirin, principally in those who are SVR to treatment there is betterment in liver histology, involving betterment in fibrosis Internationally, diagnosis and recognition of HCV infection are done on the basis of immunological assays. Among these immunological assays rapid ICT kit and ELISA are utmost common and mostly used methods. A vital problem encountered during testing is the conflict between the results of both these assays. This problem can be fixed by depending on the approach to appropriate kits. Generally, ELISA is more sensitive when compared to the rapid immune chromatographic test (ICT) kits. But ELISA test is costly. The cost of ICT kit for HCV antibody detection was reasonable. Time of reporting while using ICT is only 10-15 min but when performing ELISA time required for reporting is long. So, as ICT kits are easy to handle, take less time for reporting and as these are economically reasonable, ICT methods are more in use than ELISA in blood banks. Furthermore, now, most companies that are manufacturing ICT are consuming disease specific recombinant antigenic protein thereby specificity and sensitivity of the ICT Kits is increasing. Consuming recombinant HCV antigen, it is likely to recognize almost all persons diseased with HCV. Developing countries including Pakistan use ELISA as more sensitive and advanced technique to screen HCV. Advanced instruments and reagents are used for ELISA test. Reporting time is also prolonged so it is not easy to retest the sample for a false result. As hepatitis is highly

prevalent and result in complications like cirrhosis and cancer. Prime Minister of Pakistan introduced Hepatitis control program in collaboration of other health departments.

Two types of diagnostic tests are used for hepatitis C, most commonly. One is Immuno Chromatographic Technique and the other is Enzyme Linked Immuno sorbent Assay. ICT sometimes may show false negative or false positive test results. But results by ELISA are quite reliable and more accurate.

The current study was performed to find out the sensitivity of ELISA for Hepatitis C virus detection in serum. In addition, the objective included the comparison of ELISA with ICT for the diagnosis of hepatitis C.

2. Materials and Methods

The present study was carried out at Recep Tayyip Erdogan hospital, Muzaffargarh, Punjab, Pakistan.

Data Collection

Data was collected from the population of Muzaffargarh, area of Southern Punjab at specific time duration from June 2018 to August 2018.

Sample Size

During research work, total 200 samples were collected. From these, fifty (50) samples were collected from control group and one hundred and fifty (150) samples were from experimental group.

Control group: It was divided into two categories which include:

(i) Positive control. *These were the patients having hepatitis C. Their lab tests were confirmed positive for HCV. These patients had sign and symptoms of hepatitis C like jaundice, nausea, right upper quadrant pain, malaise, jaundice and dark urine.*

(ii) Negative control. *This group involved volunteers who had no sign and symptoms also their lab tests were confirmed negative for HCV.*

Experimental group

This group involved patients suspected of having Hepatitis C. Relevant doctor advised them to have lab tests for HCV.

Criteria for sample collection

Blood samples of patients suspected of Hepatitis C, advised by the relevant doctor.

Sampling protocol

Sample of blood (5 ml) was collected from each participant as per standard protocol of blood collection followed in pathology labs. The sample was collected after proper identification of participant by comparing participant's name, age, MR number with the request given by doctor. Then a suitable vein was chosen for veni-puncture by using a tourniquet at a site 3 to 4 inches above the vein to be punctured. The participant was asked to make a fist. When a vein was selected then needle was inserted swiftly in the vein such that needle makes an angle of 15-30 degree with the surface of arm. After collecting desired volume of blood, needle was removed and a gauze was

placed and pressed for 1 to 2 minutes to stop bleeding and to avoid hematoma formation. Then a saniplast was applied on veni-puncture site. Then blood was inserted into vacuum tube (Yellow top BD vacutainer) containing gel up to stated volume. These samples were then centrifuged and serum was obtained from each blood sample.

Chemicals and Reagents

ICT kit for anti-HCV antibody manufactured by SD Standard Diagnostics, Korea.

Vitros (ECI) immunodiagnostic system, made in USA. Ortho-Clinical Diagnostics, a Johnson and Johnson Company New York.

3. Methodology

Two Hundred blood samples; 50 samples of control group (25 positive control, 25 negative control) and 150 samples of patients (suspected for HCV) were collected. Each sample was obtained by veni-puncture and collected into vacuum tube containing gel up to stated volume. These samples were then centrifuged and serum was obtained from each blood sample. Serum was then used to test for HCV. HCV tests were performed by using ICT kit. Then these samples were tested for anti-HCV antibody through ELISA by fully automated Instrument (Vitros) and their cut off values were determined.

ICT kit Method for anti-HCV antibody

The SD BIOLINE HCV test kit, manufactured by SD Standard Diagnostics, Korea was used. ICT for HCV is a visual immunoassay to detect anti HCV antibodies qualitatively in plasma or serum of humans. Detection of antibodies is done by visually observing the developed color line on test strip. In the test region of the strip there are recombinant HCV antigens immobilized on the membrane. When test is performed these antigens form conjugate with colored particles. If in the sample, there are enough HCV antibodies then in the test region a colored line will appear. If this colored line appears in test region, then it means that test for HCV is positive. Test result is negative if this colored line does not appear. There is a control region too on the ICT membrane. Colored line in control region must appear during testing as this line shows that membrane wicking is present. It also shows that correct volume of sample is used during testing.

Test Procedure

First of all, sample and components of kit were brought to room temperature i-e 15-30 ^{0}C. Test device was placed on a flat surface. Then it was labeled with the patient's identifier number. Sample that can be plasma, serum or whole blood was poured into the specimen well on the kit labelled as S. Quantity of specimen that was taken is 10 microliters. In the next step 4 drops of sample diluent were dispensed into the specimen well. Kit was placed for 15-20 min to interpret the colored lines in the result window. Test result must not be noted after 20 min. This might yield false results. Test results were interpreted as following: **Negative Test:** If colored line appears only in control region of the kit. **Positive Test:** If colored line appears in control as well as in test region. Test kit is sensitive to both humidity as well as heat.

ELISA Test

ELISA was run on fully automated Vitros analyzer. Results of ELISA were calculated by the analyzer automatically. Cut-off values were used to interpret results. Values> 1.00 were interpreted as reactive, values <1.00

were interpreted as non-reactive. The samples included in the study were interpreted as reactive, non-reactive for HCV antibodies by ELISA. Each test run for ELISA included a kit of a negative control (NC) and positive control (PC). ELISA was performed following manufacturer's recommendations. After the ELISA was run the readings were recorded automatically. The equipment automatically validated the run and calculated the results. Interpretation of result was done based on cut-off value. If testing samples have values of absorbance less than the cut-off value, then these samples are interpreted as non-reactive for Anti-HCV. If testing samples have values of absorbance greater than or equal to the cut-off value, then these samples are interpreted as reactive for Anti-HCV.

Negative Result: Samples having absorbance less than cut off values.

Positive Result: Samples having absorbance greater than cut off values.

Kits and reagents used for ELISA should be stored at 2-8^{o}C. Likewise, ICT kit, the sensitivity and reliability of test results also depend on the storage and carefully handling of kits, chemicals and reagents used during test.

4. Results

4.1. The participants of the study

During research study at Recep Tayyip Erdogan hospital, Muzaffargarh, Punjab, Pakistan, 50 samples of control group and 150 blood samples of those people who are supposed to be suffered with hepatitis C, were collected from patients advised for (ICT and ELISA) tests for Hepatitis C.

4.2. Gender

Total 200 samples were selected for this experiment. Among these samples, fifty percent (100 samples) were collected from male and fifty percent (100 samples) were collected from females to study the effect of different gender on the sensitivity of ELISA and ICT. It is necessary to check the probability of Hepatitis C in both genders that either male have more chance to be suffered with Hepatitis C or female have more probability.

4.3. Age of patients

Age of selected patients was recorded in order to evaluate the effect of age of patients on the sensitivity of ELISA and ICT test.

4.4. Group, age and gender

Data of group, gender and age of the entire selected person was collected and shown in table 1.

Table 1. Age of male and female patients in control and experimental group

		Group (Count)	
Gender	Age	**Control**	**Experiment**
Female	Less than 20	3	16
	21-30	4	23
	31-40	9	14
	41-50	7	11
	More than 50	2	11
	Less than 20	0	10

Male	21-30	4	11
	31-40	5	17
	41-50	7	17
	More than 50	9	20
	Total	**50**	**150**

4.5. Detection of hepatitis C by ELISA and ICT method

First of all, 50 control samples were tested by both ICT and ELISA method. Fifty patients were divided in to two sub groups. Fifty percent patients were kept as positive control group and fifty percent patients were kept as negative control group. Its mean that blood sample of 25 healthy peoples and 25 hepatitis C patients were checked by both ICT and ELISA method. Results revealed that fifty percent patients suffering from hepatitis C were detected both by ELISA and ICT. Similarly, ELISA and ICT showed the negative results for all fifty percent negative control patients. It can be concluded from this experiment that both ELISA and ICT can be used for the detection of hepatitis C. Detail of 50 control patients that how many patients are male and female. ELISA showed negative results of 44 percent female patients and 56% male patients while it showed positive results of 56% female patients and 44% male patients.

4.6. Comparison between ELISA and ICT method

After evaluating the sensitivity of both ELISA and ICT method, serum samples of 150 suspected patients were tested by ELISA and ICT to evaluate that which technique give more appropriate results. Sixty eight percent patients were detected negative by both ELISA and ICT and 30.67 percent patients were detected positive by both ELISA and ICT. ELISA revealed 0.67% patient negative and ICT showed these 0.67% positive and vice versa.

Both ELISA and ICT revealed that 30.67 percent patients are suffering from hepatitis C and 68 percent patients are healthy. Results of both ICT and ELISA method are same regarding detection of hepatitis C except 1.33 percent. ICT showed positive result of a patient who is not infected by hepatitis C virus and is also confirmed as negative by ELISA. Also this patient does not show any sign and symptom of hepatitis C. Further, ICT showed negative result of hepatitis C virus infecting patient who showed the sign and symptoms of hepatitis C and also ELISA showed positive result for hepatitis C.

Table 2. Results of ELISA and ICT of male and female in control patients

		ELISA		**ICT**	
		Negative	**Positive**	**Negative**	**Positive**
Gender (count)	Female	11	14	11	14
	Male	14	11	14	11
Total		25	25	25	25
Gender (Percentage)	Female	44	56	44	56
	Male	56	44	56	44
Total		100	100	100	100

Detail of 150 experimental patients that how many patients are male and female. This shows that ICT showed false results of two female patients. 66.67 percent female patients were detected negative by both ELISA and ICT and 30.67 female patients were detected positive by both ELISA and ICT. However, 1.33 percent female patients were detected positive by ICT and negative by ELISA and vice versa.

These 150 patients are also classified according to their age. This shows that ICT give false result of which age group. This revealed that ICT give false results of patient having age group 21 to 30 years.

Table 3. Results of ELISA and ICT in experimental patients

	ICT (Count)		**ICT (Percentage)**	
ELISA	**Negative**	**Positive**	**Negative**	**Positive**
Negative	102	1	68.00	0.67
Positive	1	46	0.67	30.67
Total	150		100	

Table 4. Effect of gender on ELISA and ICT results

				ICT		**ICT (Percentage)**	
				Negative	**Positive**	**Negative**	**Positive**
Gender	**Female**	**ELISA**	**Negative**	50	1	66.67	1.33
			Positive	1	23	1.33	30.67
	Total			75		100	
	Male	**ELISA**	**Negative**	52	0	69.33	0.00
			Positive	0	23	0.00	30.67
	Total			75		100	

Table 5. Effect of age on ELISA and ICT results

Age	**ELISA**	**ICT (Count)**		**ICT (Percentage)**	
		Negative	**Positive**	**Negative**	**Positive**
Less than 20	Negative	21	0	14.00	0.00
	Positive	0	5	0.00	3.33
21-30	Negative	18	1	12.00	0.67
	Positive	1	14	0.67	9.33
31-40	Negative	24	0	16.00	0.00
	Positive	0	7	0.00	4.67
41-50	Negative	21	0	14.00	0.00
	Positive	0	7	0.00	4.67
More than 50	Negative	18	0	12.00	0.00
	Positive	0	13	0.00	8.67

4.7. Regression analysis

Effect of gender on ELISA reading

ANOVA table revealed that p value is greater than 0.05. Therefore, it's indicating that there is non-significant difference of gender on the results of ELISA. As p value is the indicator of significant or non-significant which describes either there is any significant effect of independent variable on the dependent variable or not.

Effect of age on ELISA reading

Further, regression analysis is also analysis for age on the ELISA results. Similar results were found in this case. In ANOVA table p value is also greater than 0.05 which means there is no effect of age on ELISA performance.

Effect of gender on ICT reading

In the case of ICT, same procedure is used to identify the relationship between gender and ICT performance. However, P value indicates that non-significant effect of gender on ICT performance.

Effect of age on ICT reading

Likewise, relationship of ICT performance and age was also estimated by regression and AVOVA table that indicated non-significant relationship between age and ICT.

Table 6. ANOVA table of gender on ELISA results

ANOVA[b]						
Model		Sum of Squares	Df	Mean Square	F	P value
1	Regression	.007	1	0.007	0.031	0.861[a]
	Residual	32.267	148	0.218		
	Total	32.273	149			

a. Predictors: (Constant), Gender

b. Dependent Variable: ELISA

Table 7. ANOVA table of age on ELISA results

ANOVA[b]						
Model		Sum of Squares	Df	Mean Square	F	P value
1	Regression	.157	1	0.157	0.724	0.396[a]
	Residual	32.116	148	0.217		
	Total	32.273	149			

a. Predictors: (Constant), Age

b. Dependent Variable: ELISA

Table 8. ANOVA table of gender on ICT results

ANOVA[b]						
Model		Sum of Squares	Df	Mean Square	F	P value
1	Regression	.007	1	0.007	0.031	0.861[a]
	Residual	32.267	148	0.218		
	Total	32.273	149			

a. Predictors: (Constant), Gender

b. Dependent Variable: ICT

Table 9. ANOVA table of age on ICT results

ANOVA[b]						
Model		Sum of Squares	Df	Mean Square	F	P value
1	Regression	.157	1	0.157	0.724	0.396[a]
	Residual	32.116	148	0.217		
	Total	32.273	149			

a. Predictors: (Constant), Age

b. Dependent Variable: ICT

4.8. Correlation analysis

Data of gender, age, ELISA and ICT was analyzed in pear son correlations which give the value in correlation analysis. R value indicates strength of the correlation between variables. Value of r range from -1 to +1 (0 value indicate no relation, less than 0 indicate negative relation and more than 1 reveal positive relation). Between gender vs age r value (0.230) indicate the strong relationship, negative weak correlation was indicated from the r value (0.014) between gender vs ELISA. Between gender and ICT there is also negative weak relationship which is indicated from r value (0.014). Age vs ELISA and Age vs ICT also indicate positive weak relation that is indicated by r value (0.07).

Table 10. Pearson Correlations analysis among all variables

	No. of patients	**r-value**
Gender vs Age	150	0.230
Gender vs ELISA	150	-0.014
Gender vs ICT	150	-0.014
Age vs ELISA	150	0.07
Age vs ICT	150	0.07

5. Discussion

Hepatitis C virus is a major pathogen of human being. Infection by HCV may progress to protracted hepatitis, long-lasting disease of the liver, liver cirrhosis, some auto immune diseases and hepato-cellular carcinoma (HCC). Its treatment can be difficult because it can mutate rapidly. If patient knows about the disease they are suffering from then their proper care and treatment is possible. Resulting complications can be prevented. Also transmission of this disease too the persons can be prevented. So for all this purposes, timely diagnosis of HCV infection is required.

Various lab tests are available nowadays for detection of HCV. But most commonly used lab tests are ICT and ELISA. In this study 200 samples were collected to find out the sensitivity of both these techniques. All these samples were tested by both ICT and ELISA method. Results obtained were compared to find out the technique which is more reliable and economically reasonable.

5.1. Results of ICT and ELISA for control group

Results revealed that fifty percent control persons suffering from hepatitis C (positive control) were detected by both ELISA and ICT. Similarly, ELISA and ICT showed the negative results for all fifty percent negative control persons (negative control).

5.2. Results of ICT and ELISA for experimental group

Both ELISA and ICT showed the same results regarding detection of hepatitis C in experimental group except 1.33 percent. ICT showed false positive result of a person who is healthy and also confirmed as negative by ELISA. Further, ICT gave negative result of hepatitis C infected patient who showed the sign and symptoms of hepatitis C and was also positive for HCV by ELISA. This might be due to the reason that ICT is old technique which gives qualitative results. But ELISA method is more accurate and sensitive and gives more accurate result. So, ELISA method is more reliable than ICT techniques.

5.3. Prevalence of HCV

Throughout the world HBV and HCV have attained an endemic condition. Underdeveloped countries are affected more than developed countries. In rural areas of Pakistan an alarming threat has been recorded. A huge proportion of population is affected already with HCV and HBV. Prevalence rate for HBV is 10 % and for HCV is 4-7%.

This study revealed that people who are at risk of infection by HCV include: Existing or former injection drug users, Hemo dialysis patients who are undergoing this treatment for long time, Newborns who are born to mothers infected by HCV, persons who have known exposure to HCV (for example recipients of organs or blood from a donor who were HCV positive when tested later), healthcare workers if they have needle stick injury and persons infected with HIV.

5.4. Correlation of clinical findings with ELISA and ICT results

60% of total blood samples, positive for HCV had developed jaundice. 35 % had developed non-specific symptoms including right upper quadrant pain, malaise, and nausea. 5% patients were asymptomatic but they had abnormalities in results of LFTs. This study also revealed that results of ICT and ELISA were not affected by sign

and symptoms developed by the patient. Results of both techniques are affected by antibody titer present in the sample of patient.

5.5. Need of diagnostic lab tests for HCV

The detection of anti-HCV antibodies in serum is made by different lab techniques. Two different settings are taken into account. **1**) To detect considerably infectious donations and to evade HCV infections in post- transfusion cases. Blood banks perform anti-HCV testing routinely in blood donors**. 2**) Clinical laboratories in routine examine samples for anti-HCV antibodies in patients having risk factors for viral infections acquired parenterally or in patients having clinical symptoms of HCV infection so that suitable clinical decisions as well as therapeutic decisions can be made.

5.6. Diagnostic tests used before invention of ICT and ELISA for Hepatitis C

Before the advent of ELISA tests for anti-HCV, surrogate markers such as ALT and anti-HBc were useful in identifying persons likely to transmit non-A, non-B hepatitis. Study confirms the utility of multiple ALT activity measurements in suggesting the presence or absence of HCV RNA. The vast majority of individuals negative for HCV RNA had either ALT continuously within the reference interval or only a single increased value before the time of anti-HCV testing. HCV RNA testing in such individuals is important to distinguish viremic from non viremic individuals obtained positive HCV RNA results for 92% of those with high anti-HCV titers and increased ALT in a single determination. These data suggest that there is little benefit to "confirmatory testing" in those with repeatedly increased ALT activity and high anti-HCV titers.

5.7. Benefits of ELISA test

This study showed that ELISA test is more sensitive than ICT as it showed accurate results of all the used data of 200 samples. Window period of HCV is also shortened in this method. The results of our study are in agreement with the results who revealed that the sensitivities of ELISA are 98% to 100%. Studied the sensitivity of 1^{st}, 2^{nd}, 3^{rd} and 4^{th} generation of ELISA and reported that 4th generation is based on improved specificity and sensitivity. Muthiah (2016) reported that the method which is broadly used for screening of HCV is ELISA. It is reported that both ICT and ELISA are immunological assays used for detection of HCV infection.

5.8. Limitations of ELISA test

- ELISA assessments are high-priced as the chemicals and instruments are needed for performance of test.
- Reporting time is also prolonged so it is not easy to retest the sample for a false result.

In case of ELISA, a typical micro-liter plate is used for testing so samples can be splashed from one to another well. This may interfere with the results. So there might be false positive results due to this practice.

5.9. Benefits of ICT test

- ICT kits are easy to handle.
- Time of reporting while using ICT is only 10-15min.
- It is economically reasonable.

Because of these benefits ICT methods are more in use than ELISA in blood banks. Furthermore, now, most companies that are manufacturing ICT are consuming disease specific recombinant antigenic protein thereby specificity and sensitivity of the ICT Kits is increasing. It is studied the effect of ICT techniques and concluded that rapid diagnostic ICT kits are best choice because they are not expensive and also do not require high infrastructure.

It can be concluded from this experiment that both ELISA and ICT can be used for the detection of hepatitis C. However, the sensitivity of ELISA is higher as compared to ICT. Therefore, it can be recommended that ELISA is more appropriate method for the detection of Hepatitis C.

Declarations

Source of Funding

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Competing Interests Statement

The authors declare no competing financial, professional, or personal interests.

Consent for publication

The authors declare that they consented to the publication of this research work.

Availability of data and material

The authors are willing to share the data and material according to relevant needs.

Author's Contributions

Bushra Rashid, Muhammad Ishfaq and Rida Maqbool planned and designed the research; Bushra Rashid performed the experiments; Bushra Rashid, Rida Maqbool and Muhammad Ishfaq analysed the data; Bushra Rashid & Muhammad Ishfaq wrote the manuscript.

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